Progress Report for Grant NGR-14-008-003

June 1, 1966 - November 30, 1966

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April 26, 1967

The importance of stress in its relationship to the morphologic development and functional activity of the gastrointestinal tract is emphasized primarily on lesions, secretion and motility (1,2). Studies using excessive muscular work as a stressor has been found highly effective in producing gastrointestinal ulcers and other stress syndromes such as adrenalcortical enlargement and thymus involution (3). On the other hand, lack of exercise was observed to disturb nitrogen balance, potassium and sodium metabolism, decrease the blood volume (4) and body weight*(2). The purpose of this study was to investigate whether the depressed rate and extent of growth of chronically immobilized animals was due to impaired uptake of nutrients from the gastrointestinal tract. The nutrient under study was exemplified by the use of D(-) fructose which is the common passively absorbed monosaccharide in human and lower animals (5).

MATERIALS AND METHOD

Chronic Immobilization

Male rats (Holtzman Co.) initially weighing 255±7 grams were housed individually in hanging wire cages with access to food and water ad libitum. Restrained animals were allowed space (volume = 1.037 X weight + 494) which increased linearly with body weight as well as body volume in such a way that almost complete immobilization

was achieved. The periods of restriction ranged from 5 to 25 weeks.

In Vivo

A two ml test meal of fructose-C^{14} plus fructose carrier (250 mg in water) was administered by gastric intubation to animals fasted 24 hours. Animals were sacrificed (cervical disjunction) 60 minutes after test meal administration. Ligatures were placed on the esophagus, pyloric sphincter and terminal ileum. The stomach, small and large intestine were then removed, separated, and homogenized individually in a micro-Waring blender for five minutes. The homogenates were deprotenized with Somogyi reagents, filtered, and a one ml sample of the filtrate prepared for C-14 analysis (6).

In Vitro

Unfasted animals were sacrificed and the small intestine removed and rinsed free of its contents with oxygenated mammalian Ringer solution. The everted intestinal segment technique described by Crane and Wilson (7) were followed. The intestinal preparation (10cm in length) was incubated at 37 C in Krebs-Ringer-bicarbonate solution (pH 7.4) with the test sugar added. Aerobic conditions were achieved by constantly bubbling 95% oxygen-5% carbon dioxide mixture through the incubating

medium. The initial concentration was always 200 mg/100ml of the medium; initial volume of medium on the mucosal side of the segment was 35 ml while 0.8 ml was added through the cannula to the serosal side. In general, two types of experiments using fructose-C^{14} were performed: 1) fructose medium on both the mucosal and serosal sides of the intestinal segments; 2) fructose medium only on the mucosal side and Krebs-Ringer-bicarbonate buffer without sugar on the serosal side of the everted segment.

Method of Analysis

Determination of C-14 radioactivity was achieved by counting the samples in dioxane-naphthalene solvent with liquid scintillation spectrometer. In vitro intestinal segments were solubilized in NCS-1 reagent (Nuclear Chicago) prior to counting. In vitro fluid volume was calculated as total dpm in solution/dpm per ml solution.

RESULTS

In Vivo

Radiochromatographs of the recovered material (deprotenized with TCA) show the C-14 to be confined to a single peak; therefore fructose absorbed (Table 1) was assumed to be fructose administered minus fructose recovered from the gastrointestinal tract at the end of the 60 minute absorption period.

The regression line of control animals from

5th to 25th week of experimental housing shows no change (Figure 1). Over the same period the regression line of the restrained group shows a significant slope ($P < 0.01$). Further variance analysis demonstrates that the regression line for restrained animals differed significantly ($P < 0.05$) from the unrestrained group.

In Vitro

When fructose-C^{14} was initially present on both sides of an everted rat intestine, the mucosal fructose concentration was found to be greater than the serosal fructose concentration after 60 minutes incubation (Table 2). After incubation recovery of fructose (mg) from mucosal solution was shown to be considerably less than that initially added; however, there was no significant change in the amount of fructose present in the serosal solution. An increase in final serosal volume occurred as the result of water movement across the wall from the mucosal side. At the same time, the mucosal fluid volume was slightly decreased from the initial volume.

When fructose was placed only in the mucosal medium, there was a movement of fructose across the wall into the serosal solution with a concomitant movement of fluid in the same direction. Although no significant difference in fluid or fructose movement was observed between groups, more fructose was moved than when both

compartments were initially filled with fructose medium.

The segment was also shown to permit sugar movement from serosal to mucosal solution when only the serosal medium contained fructose initially. However, the net fluid movement in the system always remained unidirectional, that is from mucosal to serosal solution.

Concentrations of both serosal and mucosal solutions did not differ significantly between control and restrained or between 5th and 25th weeks (Table 3). Movement of water from the serosal to mucosal solutions was shown to be greater in the 25th week than in the 5th week animals; however, the intestinal segments of both control and restrained animals contained from 31-52% less fructose per gram tissue fluid in the 25th week than comparable segments from animals in the 5th week.

Results of a study of 80 oven-dried intestinal segments from both groups of five week animals indicate that $80 \pm 1\%$ of the wet weight is tissue fluid and that there is also no difference in per cent dry weight between groups (control versus restrained).

DISCUSSIONS

The present method using physical confinement as a stressor agent on rats over a period of 25 weeks has demonstrated, at the end of one week, a significant body weight depression which continues throughout the 25 weeks.

The effects of other experimental situations which might be considered stressful have been reviewed. Controversial results on body weights of chronically restrained animals have been reported (8,9). These findings have, however, strongly suggested that not only the length and severity of restriction which had been used in various experiments but also the biological variations of the organism - species, age etc. - at time of exposure could be of utmost importance in eliciting stress responses.

In vivo absorption studies at periods of 5, 10, 15, 20 and 25 weeks of immobilization showed no change in unrestrained animals over these periods. During the same intervals fructose absorption were significantly greater with restraint. However, the modification in the ability of restrained animals to transport and/or absorb fructose, suggests prolonged restraint causes either a stimulatory effect on greater gastrointestinal uptake or a change in metabolic utilization of fructose.

In vivo, the smaller quantity of unabsorbed fructose recovered from the intestinal tract of restrained animals appears to indicate a greater fructose absorption over the 25 week period; but, comparison of in vitro results has shown no significant changes in any of the parameters of the system between groups (restrained versus control) at 5th and 25th weeks. Thus, the failure to demonstrate any deviations in fructose absorption between groups would

seem to account for the multiple interrelationships associated with the more widely involved stress responses (1) part of which the *in vitro* preparation was not able to reproduce.

The present study suggests that the application of both *in vitro* and *in vivo* methods offers a useful approach to the elucidation of gastrointestinal absorptive function during chronic restraint. The exact mechanism of increasing fructose absorption *in vivo* when the rats were restrained is not yet known; however, with additional *in vitro* experiments, it is hoped that the functional shifts in gastrointestinal tract due to stress will be better understood.

TABLE 1. Fructose Absorption *In Vivo*

Weeks †	Stomach mg Fructose Recovered		Fructose Absorbed, mg *	
	Control	Restrained	Control	Restrained
5	16.93±5.60	9.82±2.05	116.26±10.11(9)	118.34±8.77(10)
10	12.60±2.11	23.13±8.01	111.27±8.78(10)	114.83±7.80(10)
15	19.08±3.96	15.07±3.92	112.69±5.25(10)	121.64±6.23(10)
20	22.15±3.92	28.12±5.41	105.08±4.23(10)	120.24±3.47(9)
25	16.34±4.41	16.54±8.64	132.00±6.43(9)	153.12±10.66(9)

* Fructose absorbed = fructose administered minus amount recovered. Values represent means in mg±SE. Number of animals in parentheses.

† Weeks of chronic immobilization.

TABLE 2. *In Vitro* Fructose Movement At 5 Weeks

		Mucosal, mg/100 ml	Serosal, mg/100 ml	Intestine, mg Recovered	Serosal Volume ml Increased
Control(11)	I*	200.0	200.0		
	F	197.0±0.4	173.4±3.2	1.39±0.06	0.03±0.03
Restrained(11)	I	200.0	200.0		
	F	196.2±0.7	178.1±6.5	1.39±0.09	0.12±0.03
Control(12)	I	200.0	0.0		
	F	199.7±1.2	73.6±7.5	1.36±0.11	0.09±0.02
Restrained(11)	I	200.0	0.0		
	F	195.7±1.1	84.8±5.5	1.15±0.08	0.08±0.01
Control(6)	I	0.0	200.0		
	F	1.21±0.02	106.5±2.8	0.29±0.01	0.03±0.01
Restrained(6)	I	0.0	200.0		
	F	1.30±0.01	101.2±4.7	0.28±0.02	0.07±0.01

* I = before incubation. F = after 60 minute incubation period. Values represent means±SE.

TABLE 3. *In Vitro* Fructose Movement At 25 Weeks

		Mucosal, mg/100 ml	Serosal, mg/100 ml	Intestine, mg Recovered	Serosal Volume ml Increased
Control(10)	I *	200.0	200.0		
	F	193.8±1.01	161.5±5.24	0.93±0.04	0.15±0.03
Restrained(10)	I	200.0	200.0		
	F	195.5±0.88	163.3±3.67	0.78±0.07	0.15±0.03
Control(8)	I	200.0	0.0		
	F	192.3±1.38	64.0±4.08	0.66±0.03	0.15±0.03
Restrained(8)	I	200.0	0.0		
	F	191.7±0.90	71.7±5.16	0.67±0.03	0.13±0.03

* I = before incubation. F = after 60 minute incubation period. Values represent means±SE.

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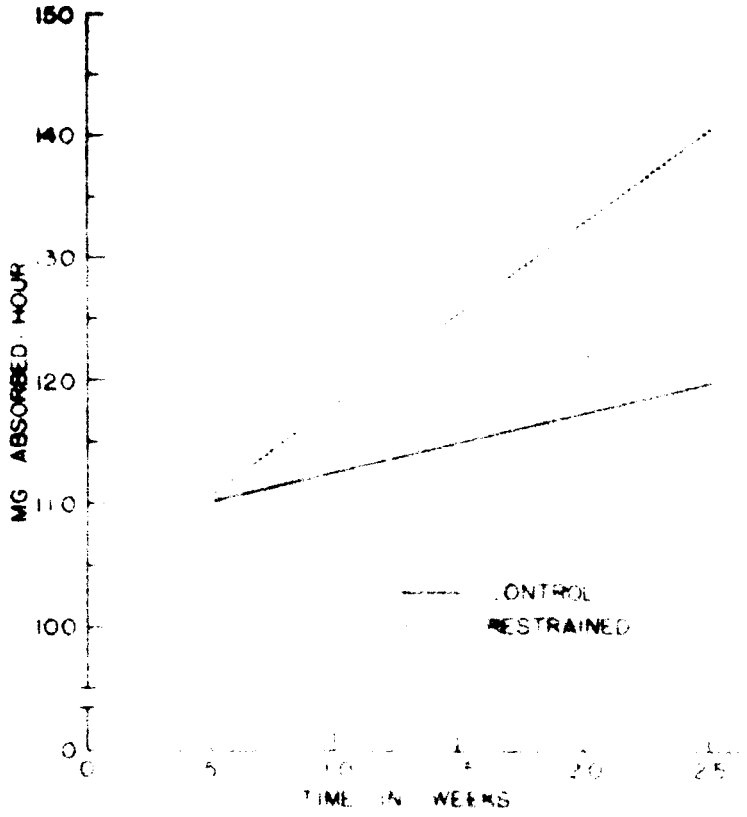
LEGENDS FOR ILLUSTRATIONS

FIGURE 1.

Regression lines, giving mg of fructose absorbed per hour *in vivo* as a function of weeks of experimental housing (5,10,15,20 and 25).


150
140
130
120
110
100
0
MG ABSORBED HOUR
CONTROL
RESTRAINED
0
5
10
15
20
25
TIME IN WEEKS